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13. ABSTRACT *(Maximum 200 words)*

The overall goals of this project were to analyze various processes contributing to the generation and control of behavior, and to study mechanisms underlying modification of behavior by learning and memory. The investigation focused on feeding behaviors of marine mollusk *Aplysia* and on neurons that generate these behaviors. Progress was made in five areas: 1) The investigation of mechanisms of switching from rejection to ingestion of food has revealed that neuron B4/5 is involved in biasing the feeding center toward generating ingestion. 2) Analysis of modulation of feeding by the neurotransmitter dopamine indicated that it has various and cell-specific effects on different neurons controlling feeding. The changes appear to synergistically reconfigure the neurons to generate ingestion. 3) In the study of learning during operant conditioning, neuron B51 was found to be associated with the expression of ingestion motor patterns. B51 appears to be sufficient for the contingent-dependent enhancement of the ingestion-like activity. 4) Pharmacological analysis suggested that dopamine is involved in the contingent-dependent modulation of feeding. 5) To study the mechanisms of classical (Pavlovian) conditioning, a procedure for conditioning of feeding behavior was developed.

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Final Technical Report

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I. Objectives:

There have been no changes in the objectives of this project.

II. Status of Effort

The overall goals of this project were: 1) to analyze molecular, cellular and network processes contributing to the generation and control of behavior, and 2) to analyze neural mechanisms underlying modification of behavior by learning and memory. The investigation focused on feeding behaviors of marine mollusk *Aplysia* and on neurons, located in the buccal ganglia of *Aplysia*'s brain, that generate these behaviors. Progress was made in five areas: 1) The investigation of neuronal mechanisms of switching from rejection to ingestion of food has revealed that certain changes in properties and connections of multifunctional neurons B4/5 are associated with biasing the buccal neurons toward generating ingestion-like buccal motor programs (BMPs). 2) Analysis of modulation of feeding by the neurotransmitter dopamine indicated that it has various and cell-specific effects on different neurons that constitute the neuronal ensemble (circuit) controlling the feeding. The changes appear to work in synergy to reconfigure this circuit to favor the generation of ingestion-like BMPs. 3) In the study of learning during operant conditioning, i.e., when reinforcement is contingent upon a particular behavior (e.g., ingestion), the activity in another neuron, B51, has been found to be associated with the expression of ingestion-like BMPs. The synaptic and cellular properties of B51 were tested that could be specifically associated with this pattern. B51 appears to be sufficient for the contingent-dependent enhancement of the ingestion-like pattern. 4) Pharmacological analysis of the neural pathway that mediates contingent-dependent modulation of feeding toward the increase in ingestion-like responses suggested that dopamine is involved in this modification. 5) To study mechanisms of learning during classical (Pavlovian) conditioning, a procedure was developed that produced conditioning of feeding behavior.

III. Accomplishments/New Findings

During previous years of AFOSR sponsored research, this laboratory investigated modifications that can be introduced in the neurons generating feeding by various factors, as

motivational state, modulatory transmitters, and learning. Application of the transmitter dopamine, activation of dopaminergic neuron B65, or reinforcement of a specific pattern of neuronal activity in an isolated brain preparation all resulted in biasing feeding motor programs from rejection-like toward ingestion-like activity. This change was characterized by a shift in the phase distribution of activities of many neurons involved in buccal patterned activity. During the past funding period, these studies began to investigate cellular and pharmacological mechanisms that underlie the changes in the phase distribution of neuronal activity, and the plastic changes that occur in the nervous system during changes from one adaptive behavior to another.

A. Involvement of neuron B4/5 in biasing the buccal CPG toward ingestion-like fictive feeding in *Aplysia*.

Consummatory feeding in *Aplysia* consists of different behaviors such as rejection and ingestion. These behaviors occur due to complex stereotypic movements of the feeding organs. An ensemble (neural circuit) of neurons controlling these rhythmic movements is located in the buccal ganglia of *Aplysia*'s central nervous system. This circuit constitutes a central pattern generator (CPG) that produces bursts of patterned activity, which, in turn, drives motor neurons controlling muscles of the feeding organs. As a result, the system sends an output signal to muscles in the form of rejection- or ingestion-like buccal motor programs (BMPs) that mediate coordinated feeding movements. A switch from rejection- to ingestion-like BMP occurs for extended periods of time when animals eat or when ganglia are perfused with the neurotransmitter dopamine (Kabotyanski *et al.*, 1994). The key to identifying either of the BMPs is the activity of motor neurons (e.g., B8A/B) that control closure of radula, the organ that grabs the food (seaweed). During rejection, the radula closes as it protracts, whereas during rejection-like BMPs, neurons B8 fire in the protraction phase (Fig. 1A). During ingestion-like BMPs, the B8 activity shifts so that it occurs in phase with retraction (Fig.1B). The neuronal mechanisms of this phase shift are currently unknown. One neuron that may be involved in such phase shifts is B4/5. Its firing rate is decreased during the retraction phase of ingestion-like fictive feeding induced by dopamine (Fig.1B; see also Kabotyanski *et al.*, 1994) or by newly identified neuron B65 (Kabotyanski *et al.*, 1998), and B4/5 elicits IPSPs in B8A/B.

A series of experiments were performed to test the hypothesis that a suppression of neuron B4/5 activity during retraction may contribute to an increase of B8A/B activity during retraction, which biases the buccal CPG toward producing ingestion-like fictive feeding.

Intracellular recordings were made in isolated buccal ganglia. First, it was demonstrated that firing in B4/5 could reduce or interrupt spiking in the neuron B8A/B. The next step examined whether inhibition of neuron B4/5 could affect the phase distribution of activity in neuron B8A/B. Normally, preparations exhibited spontaneous rejection-like activity (Fig. 2A). When one B4/5 was hyperpolarized at the onset of the retraction phase, significantly more spikes in both ipsilateral neurons B8A/B occurred during the retraction phase (Fig. 2B). Rejection-like patterned activity was restored if B4 was not hyperpolarized (Fig. 2C). In a separate set of experiments, both B4 and B5 neurons ipsilateral to B8A/B were hyperpolarized to prevent their activity. This manipulation led to a more pronounced shift in B8A/B activity toward the retraction phase.

These results indicate that inhibition of neuron B4/5 redistributes the activity of neuron B8A/B toward the retraction phase, and suggest that B4/5, possibly in concert with B51 (Nargeot *et al.*, 1997), may be involved in switching between rejection-like and ingestion-like BMPs.

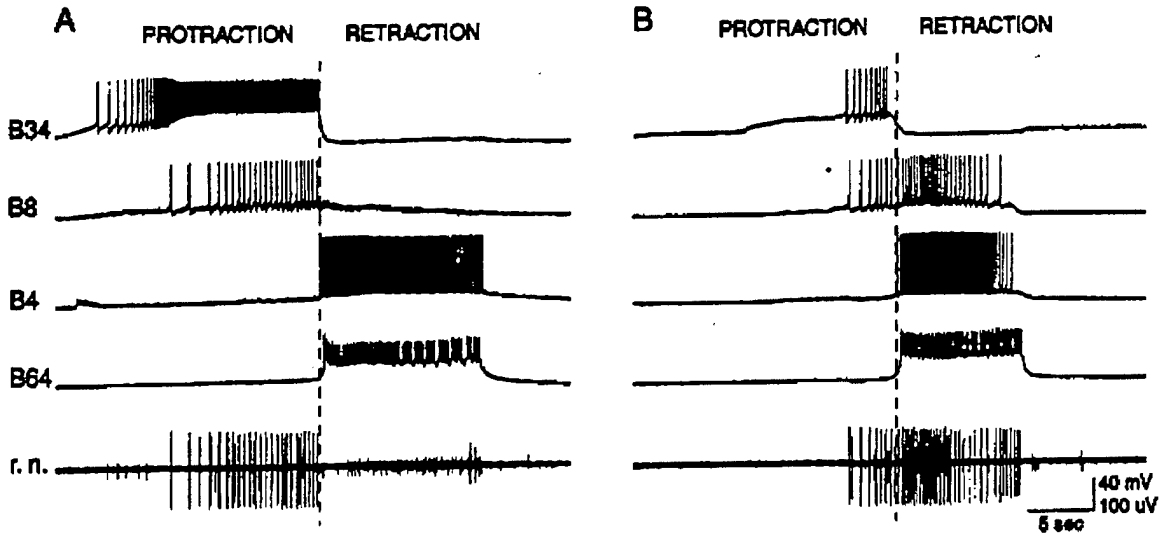

Figure 1. Intracellular recordings of patterned activity in neurons of the feeding CPG during different BMPs. *A*: usually, isolated buccal ganglia exhibit rejection-like BMPs. These BMPs are characterized by activity in B8 coinciding with protraction phase of the cycle and by a high level of activity in B4/5. *B*: ingestion-like BMP in presence of dopamine. Most of activity in neuron B8 occurs during retraction phase, and the level of activity in neuron B4/5 is reduced.

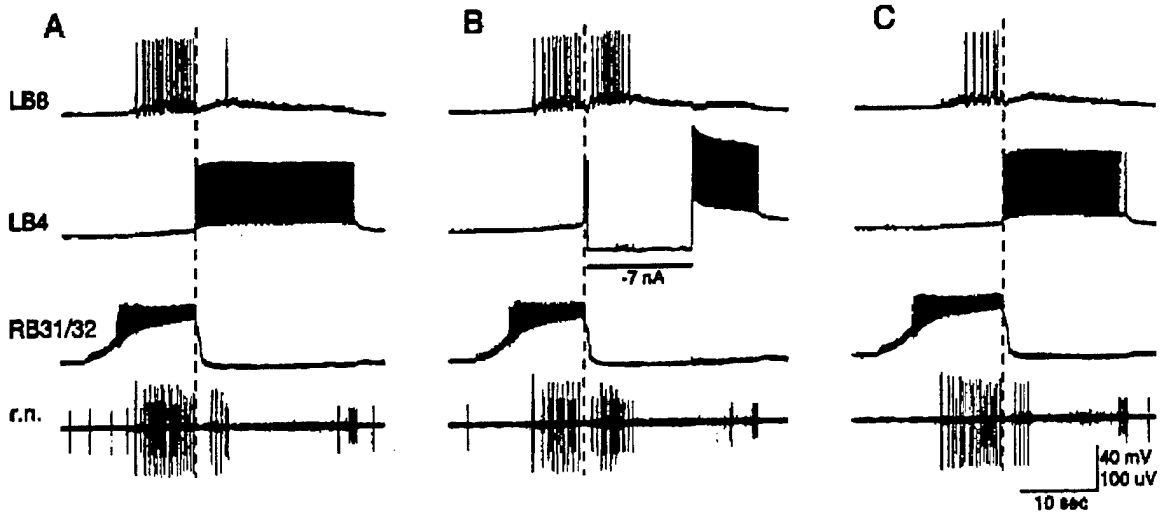

Figure 2. Inhibiting activity in neuron B4/5 during retraction phase concurrently increases activity in ipsilateral B8. *A* and *C*: spontaneous rejection-like BMPs. *B*: B4 was hyperpolarized at the onset of the retraction phase causing the increase of B8 activity during retraction, which was indicative of intermediate BMP.

B. Cellular and synaptic analysis of dopamine-induced modulation of fictive feeding

Dopamine (DA) is an important neuromodulator of feeding in *Aplysia*. Previously, this laboratory has found that DA is involved in activation of ingestion in semi-intact preparations (i.e., containing brain and peripheral organs). DA also elicited ingestion-like BMPs, or fictive feeding, in isolated buccal ganglia (Kabotyanski *et al.*, 1993-1995, 1998). Thus, the next step was to examine cellular and synaptic changes that may underlie the modulatory effects of DA.

Intracellular recordings were made from identified neurons in the isolated buccal ganglia. The protraction or retraction phases of a BMP were monitored via recordings from identified neurons known to be active during either protraction or retraction (retraction-group cells) of the radula. The type of a BMP was assessed based on the timing of activity in radula closure motor cells B8A/B relative to retraction phase (see above). In control conditions (perfusion with artificial seawater), the buccal ganglia exhibited low level of spontaneous BMPs. These BMPs were mostly rejection-like (Fig. 1A). Perfusing the preparation with DA (50 μM) gradually increased the frequency of spontaneous BMPs after 1 hour of application. By this time, most BMPs were ingestion-like (Fig. 1B). Firing rate increased in neuron B8, but it gradually decreased in neurons B34 and B63. Retraction-group neurons B4/5 exhibited significantly decreased rate of activity. In the presence of DA, depolarization was observed in protraction-group neurons B31/32, B34 and B65, and hyperpolarization was observed in B63. Retraction-group neurons B4/5, B51 and B64 were hyperpolarized. B51 become unable to produce a regenerative burst during retraction. Other electric properties, such as input resistance, excitability or post-inhibitory rebound were differently changed in different cells. In addition, DA specifically affected the strength of some synaptic connections. These effects were usually reversible by washout for 1 hour. The metabolic precursor of dopamine L-DOPA (200 μM) had similar effects except that they were delayed by 15-20 min.

These data indicate that DA has various and cell-specific effects on different elements of the feeding circuit. The changes appear to work in synergy to reconfigure the network to favor the generation of ingestion-like BMPs. For example, the decrease of firing in neuron B34, a cell believed to be critical for rejection-like BMPs, would favor the expression of ingestion-like patterns in presence of DA. On the other hand, the decrease of excitation from B64 to B4/5 and decrease of excitability of B4/5 could account for the reduced firing in neuron B4/5 during retraction. As a result, the network becomes biased for the production of ingestion-like BMPs.

C. Role of neuron B51 in the expression of the ingestion-like patterns

Neuron B51 is an element of the buccal CPG that is presynaptic to motor neurons recruited during ingestion. Previously, B51 was found in this laboratory to fire primarily during the retraction phase of ingestion-like BMPs and primarily silent during the rejection-like BMPs. When active during ingestion-like BMPs, activity of B51 was associated with key features of this BMP such as the radula closure activity during the retraction phase and prolonged duration of the retraction phase. Neuron B51 produced strong monosynaptic EPSPs in the closure motor neurons B8 and in the retraction interneuron B64. Experimental manipulations of activity in B51 during ongoing BMPs significantly changed the occurrences of ingestion-like BMPs. The occurrences of this type of BMPs were enhanced when B51 was forced to fire during all types of BMPs. In contrast, the occurrences of ingestion-like BMPs decreased when activity of B51 was prevented.

These results indicated that B51 participate in the expression of ingestion–like BMP, and that the dynamics of activity in B51 could mediate the recruitment of neuronal activity that accompany the transition between different BMPs.

D. Neuronal mechanisms for selective enhancement of the ingestion-like BMP by an analogue of operant conditioning

Consummatory feeding behaviors can be modified by different associative learning procedures such as operant conditioning and classical conditioning. In operant conditioning, the contingency between reinforcement and a designated behavior selectively changes the frequency of occurrences of this behavior. In order to investigate the cellular mechanisms of operant conditioning, the neuronal modifications induced by an *in vitro* analogue of this conditioning in the buccal ganglia were examined in this study.

Three groups of preparations were used: contingent reinforcement, yoke-control and control groups that differed by the events occurring during the training protocol. In the contingent reinforcement group, reinforcement (i.e., stimulation of the esophageal nerve) was contingent upon the ingestion-like BMP. In the yoke-control group, the reinforcement was delivered but was not contingent to any ongoing BMP. In the control group, no reinforcement was delivered. After training, the occurrences of ingestion-like BMP significantly and selectively increased in the contingent-reinforcement group as compared to the yoke-control or the control group (Nargeot *et al.*, 1997). In these preparations, the synaptic and cellular properties of cells that are specifically associated with this BMP were also tested. Comparison of activity in B51 among the different groups of preparations demonstrated that the frequency of occurrences of this activity was significantly increased in the contingent reinforcement group as compared to the yoke-control or control groups. This modification of the functional dynamics of B51 was associated with an enhancement of the input resistance and the excitability of B51.

Another set of experiments examined whether activation of B51 can mediate the selective enhancement of the reinforced BMP. A training paradigm similar to that described earlier was used. However, the activity of B51 rather than BMPs was explicitly conditioned. In the contingent-reinforcement group, reinforcement was contingent upon experimental depolarization of B51. In the yoke-control group, reinforcement was not contingent upon the depolarization of B51. In the control group, no reinforcement was delivered. The input resistance and excitability of B51 was enhanced in the contingent reinforcement group as compared to either control. These modifications in B51 were associated with a modification of the activity of B51. This cell was more frequently active in the contingent reinforcement group as compared to the yoke-control or control groups. Finally, comparison of the ongoing BMPs among the different groups demonstrated that contingent-reinforcement of activity in B51 subsequently enhanced the occurrences of ingestion-like BMP as compared to the yoke-control or control groups. No change in the other patterns (e.g., rejection-like BMP) was observed among the different groups.

These results suggest that contingent-dependent modifications of BMPs can result from the modification of the functional recruitment of a pattern-specific cell. This modification can explain an essential feature of operant conditioning that is the selective enhancement of the reinforced operant.

E. Pharmacological analysis of the role of dopamine in contingent-dependent enhancement of the ingestion-like BMP

In the analogue of operant conditioning, the stimulation of the esophageal nerve (E n.) was used as an analogue of reinforcement. Previous studies have found that E n. contains catecholamine-rich fibers. Moreover, catecholamines such as dopamine modulated rhythmic activity in the buccal ganglia so that ingestion-like BMPs are produced (see above; Kabotyanski *et al.*, 1998). Thus, the role of dopamine in contingent-dependent enhancement of the ingestion-like patterns was investigated.

Two groups of preparations were examined: contingent reinforcement and yoke-control groups. In the contingent stimulation group, stimulation of E n. was contingent upon ingestion-like BMP. In the yoke-control group, the same stimulation of E n. was used, but this stimulation was not contingent with any BMPs. Half of the preparations of each group were bathed in a control solution (ASW), the other preparations were bathed in ASW containing the dopamine antagonist, methylergonovine [10^{-5}M]. In accordance with previous findings, in ASW there was a significant increase in ingestion-like patterns between contingent-reinforcement and yoke-control groups. In contrast, in methylergonovine there was no significant difference between groups. Moreover, there was no significant difference in other BMPs (e.g., rejection-like BMP) between contingent-reinforcement and yoke-control groups using either ASW or methylergonovine. Because contingent reinforcement modified the cellular properties of B51, this study has begun to investigate whether the esophageal nerve makes dopaminergic synapses on B51. In saline rich in divalent ions that suppress polysynaptic activity, stimulation of esophageal fibers was found to elicit a postsynaptic potential in B51. This monosynaptic connection was blocked by addition of methylergonovine in the bath.

These findings indicate that a dopamine antagonist can suppress the increase in ingestion-like responses that accompany associative training without altering the activity of other patterns. They suggest that dopamine may be one neurotransmitter involved in modifications of ingestion-like BMPs, and possibly of neuron B51, which were induced by an analogue of operant conditioning.

F. Classical conditioning of feeding behavior in *Aplysia*

To allow the extension of cellular analysis of plasticity in *Aplysia* during appetitive forms of learning, a classical conditioning procedure was developed that involved feeding behavior. Conditioning was demonstrated in two independent experiments that differed slightly in design.

In the first experiment, animals were conditioned by pairing a weak tactile stimulus applied by a glass probe to the lips (conditioned stimulus, CS) with food (unconditioned stimulus, US). Two blocks of training were separated by a 30 min rest period. The control group received CS and US specifically unpaired. Retention was tested blind at 1 hr after training by presenting the CS. The frequency of bites elicited by this test CS was compared to a baseline frequency before the onset of stimulation. The increase in the frequency of bite responses after CS presentation in the paired group was significantly greater than that in the unpaired group.

The second experiment differed as follows: a paint brush was used as CS; there was no rest period between trials; and the naive response of each animal was tested by applying the CS to the lips of the animal before training. The number of bites to the presentation of four CSs before, and 1 hr after, training was compared. Animals in the paired group responded to the test

CS with statistically significant increase in the number of bites, unlike animals in the unpaired group. A second set of animals was trained using this protocol to test for long-term (24 hr) retention of classical conditioning. Again, animals that received paired training showed a greater number of bites to the CS at the 24 hr time point than animals that received unpaired training. These results indicate that feeding behavior in *Aplysia* can be classically conditioned and that the memory for this conditioning persists for at least 24 hours.

The behavioral plasticity demonstrated here is ideally suited for further analysis on a cellular level since many of the components of the CPG that control feeding are known. Furthermore, feeding behavior can be operantly conditioned. Thus, an analysis of associative plasticity in this system would allow for a direct comparison between mechanisms for operant and classical conditioning.

G. Summary and conclusions

These studies have begun to uncover specific cellular and synaptic mechanisms by which the neural circuit for feeding may control switching between two behaviorally opposite rhythmic motor programs (i.e., ingestion and rejection). For example, depression of activity in neurons B4/5, or activation of B51 result in ingestion-like patterns. Furthermore, the cellular and pharmacological mechanisms of an associative form of learning – operant conditioning – were examined. Moreover, the pharmacological analysis of the contingent-dependent plasticity linked the study of operant conditioning with another line of this laboratory's research concerned with mechanisms by which the modulatory neurotransmitter dopamine could reconfigure the feeding network. It appears that mechanisms involved in dopamine- or contingent-dependent modifications of feeding represent some fundamental principles of plasticity that are employed by the neuronal network for feeding during its behavioral adaptations. The development of a classical conditioning analogue will help to expand the understanding of specific and general principles of different forms of associative modifications of behavior.

IV. Personnel Supported

Baxter, Douglas A., Ph.D.	Assistant Professor - Research
Herynk, Kara J.	Research Assistant
Kabotyanski, Evgeni A., Ph.D.	Postdoctoral Research Fellow
Lechner, Hilde	Graduate Student
Nargeot, Romuald, Ph.D.	Postdoctoral Research Fellow
Patterson, Gary W.	Undergraduate / Research Assistant

V. Publications

A. Articles

Nargeot, R., Baxter, D.A. and Byrne, J.H. Contingent-dependent enhancement of rhythmic motor patterns: an in vitro analogue of operant conditioning. *J. Neurosci.* 17(21): 8093- 8105, 1997.

Kabotyanski, E.A., Baxter, D.A. and Byrne, J.H. Identification and characterization of catecholaminergic neuron B65 that initiates and modulates patterned activity in the buccal ganglia of Aplysia. *J. Neurophysiol.* 79: 605-621, 1998.

B. Chapters

Byrne, J.H. Invertebrate models of learning. In: *The Encyclopedia of Neuroscience*, Second Edition, eds. Adelman, G. and Smith, B.H., Elsevier Science, Amsterdam, in press.

C. Abstracts

Kabotyanski, E.A., Baxter, D.A. and Byrne, J.H. Involvement of neuron B4/5 in biasing the buccal CPG toward ingestion-like fictive feeding in *Aplysia*. *Soc. Neurosci.* Abstr. 23:1046, 1997.

Lechner, H.A., Baxter, D.A. and Byrne, J.H. Classical conditioning of feeding behavior in *Aplysia*. *Soc. Neurosci.* Abstr. 23:1334, 1997.

Nargeot, R., Baxter, D.A. and Byrne, J.H. Dynamic recruitment of neurons into the central pattern generator (CPG) for feeding is associated with motor pattern generation selection in *Aplysia*. *Soc. Neurosci.* Abstr. 23:1046, 1997.

Baxter, D.A., Nargeot, R., Patterson, G.W. and Byrne, J.H. A dopamine antagonist (ergonovine) impaired the enhancement of motor patterns by contingent reinforcement in the isolated buccal ganglia of *Aplysia*. *Soc. Neurosci.* Abstr. 24, 1998 (submitted).

Nargeot, R., Baxter, D.A. and Byrne, J.H. Contingent reinforcement in an analogue of operant conditioning modified the intrinsic membrane properties of an identified *Aplysia* neuron (B51). *Soc. Neurosci.* Abstr. 24, 1998 (submitted).

Kabotyanski, E.A., Baxter, D.A. and Byrne, J.H. Cellular and synaptic analysis of dopamine-induced modulation of fictive feeding in *Aplysia*. *Soc. Neurosci.* Abstr. 24, 1998 (submitted).

D. Manuscripts in Preparation

Kabotyanski, E.A, Baxter, D.A., Cushman, S.J., Nargeot, R., and Byrne, J.H. Modulation of fictive feeding in *Aplysia* by dopamine and serotonin. *J. Neurophysiol.* (in preparation)

Nargeot, R., Baxter, D.A. and Byrne, J.H. *In vitro* analogue of operant conditioning. I. Contingent reinforcement modifies the functional dynamics of an identified neuron. (in preparation)

Nargeot, R., Baxter, D.A. and Byrne, J.H. *In vitro* analogue of operant conditioning. II. Modifications of the functional dynamics of neuron B51 mediate motor pattern selection. (in preparation)

VI. Interactions/Transitions

A. Participation/Presentations

Baxter: Invited speaker, Conference on Cell & Molecular Biology of *Aplysia* and Related Invertebrates, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1997.

Byrne: Invited discussant, the 80th Dahlem Conference on the Mechanistic Relationship between Development and Learning: Beyond Metaphor, Berlin, 1997.

Byrne: Invited speaker, the Eighth Annual Spring Brain Conference, Sedona, Arizona, 1997.

Byrne: Conference Co-organizer, the Fifth International Meeting on the Cell and Molecular Biology of *Aplysia* and Related Invertebrates, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1997.

Byrne: Invited speaker, NIH Conference on Control of Genes, Development and Plasticity by Neural Impulses, Bethesda, Maryland, 1997.

Byrne: Invited participant, Air Force Office of Scientific Research Chronobiology & Neural Adaptation Program Review in Colorado Springs, Colorado, 1997.

Byrne: Invited participant, workshop on Human Cognition and How It Fails, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1997.

B. Consultative and Advisory Functions

Byrne: Member-at-Large, Section Committee of the Section on Neuroscience, American Association for the Advancement of Science, 1996-present.

Byrne: Member, Special NIH Study Section on Genetics, 1997.

Byrne: Member, Advisory Committee, John Sealy Memorial Endowment Fund for Biomedical Research, 1994-1997.

Byrne: Member, Scientific and Academic Advisory Committee, Weizmann Institute of Science, 1997.

VII. New Discoveries, Inventions or patent Disclosures

None.

VIII. Honors/Awards

Byrne: NIMH Research Scientist Award, 1993.